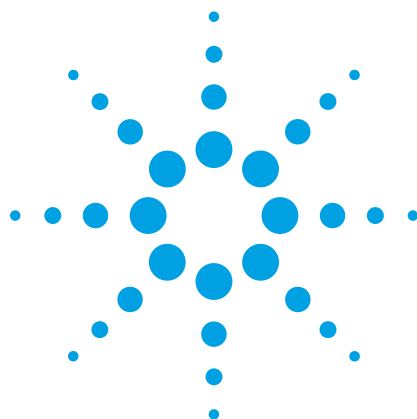


Macroporous Reversed-Phase C18 High-Recovery Protein Fractionation HPLC Column

Another tool from Agilent increases certainty in protein biomarker discovery

Find the Right Path Sooner

Agilent is working to help you improve the quality of life with tools that provide more certainty, more simplicity, more confident results in less time, and more possibilities to achieve your goals.



4.6 x 50 mm mRP-C18 column (part number 5188-5231). A proprietary surface treatment, in addition to the column's macroporous (large-pore) bonded-silica material, prevents irreversible protein adsorption.

High-Recovery Protein Fractionation and Desalting

In the past, HPLC columns have not been the choice for protein fractionation because of low protein recovery. Proteomics scientists, therefore, use gel electrophoresis or other time-consuming or irreproducible techniques.

Now there's a better choice. The Agilent Macroporous Reversed-phase C18 (mRP-C18) High-recovery Protein Fractionation Column offers **greater than 95 percent protein recovery**. In fact, greater than 98 percent recoveries have been observed with immunodepleted serum.

mRP-C18 Column Benefits

- **Remarkably high recovery** of intact proteins, including high- and low-molecular weight species.
- **Fast desalting.** Eliminates the need for lengthy spin concentration for desalting (normally a three-hour process) after immunodepletion of high-abundant proteins. This column lets you desalt in about 13 minutes.
- **Your choice in number of fractions.** This column and method allow for reproducible desalting and separation of complex protein samples (such as immunodepleted serum or plasma proteins) into the number of fractions you want—for easy and

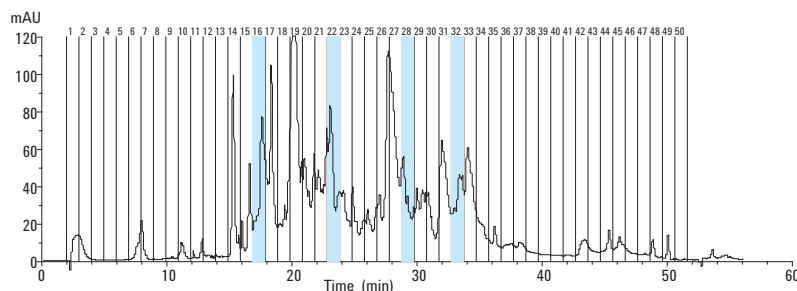
consistent protein fractionation. This is particularly useful for biomarker discovery.

- **Three times more loadability** (380 µg) than conventional HPLC C8 columns and other reversed-phase packings.
- **High resolution and selectivity** for large-molecule analytes (proteins). Proteins remain in tight bands, isolated to the fractions of interest.
- **Reduced sample complexity** prior to downstream analyses like gel electrophoresis and LC/MS.

Simple protocol chemistry.

Simple mobile phases and gradient—used with standard HPLC equipment—make protein fractionation easy and reproducible.

- **Reusability** for efficient fractionation and for processing many samples.
- **Compatible** with the Agilent 1100 Series LC system, standard HPLC equipment, and reversed-phase mobile phases.



Sample	Number of Proteins Identified
Human Serum*	40
Immunodepleted Human Serum**	181
Fraction 16	71
Fraction 22	97
Fraction 28	99
Fraction 32	181
Combined Fractions (16, 22, 28, 32)	394

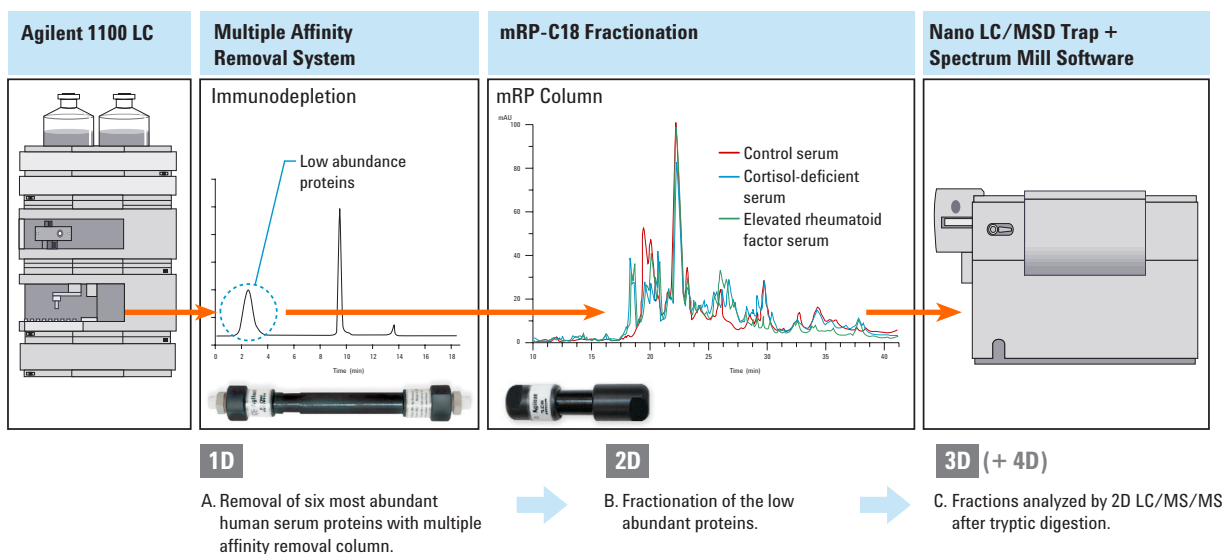
Immunodepleted human serum in 50 separate fractions. After using the mRP-C18 column, Agilent chemists used LC/MS/MS to identify 10 times more proteins (approximately 400) in just 4 out of the 50 fractions than when analyzing untreated crude serum without the mRP-C18 column and immunodepletion.

* Human serum data collected off-line by 2D LC/MS/MS.

** Immunodepleted with the Agilent Multiple Affinity Removal System.



Agilent Technologies



Protein biomarker workflow using the Agilent Multiple Affinity Removal System, the mRP-C18 Column, LC and MS instrumentation, and software. Using this workflow for immunodepleted serum, the mRP-C18 column helps to clearly identify differences among control serum, cortisol-deficient serum, and elevated rheumatoid factor serum, as shown here. This simple visual procedure allows you to quickly choose which fractions to study for comparing different serum samples for potential biomarkers.

Perfect Partners Reduce Sample Complexity

Having Agilent's **Multiple Affinity Removal System** columns or spin cartridges eliminate targeted high-abundance interfering proteins prior to using the **mRP-C18 column** avoids overloading the column or blocking column surfaces with albumin. Low-abundance fractions can be loaded onto the mRP-C18 column with minimal sample preparation, reducing the chance of losing proteins. This frees you to concentrate on low-abundance biomarkers as you dig deeper into the proteome.

More Possibilities to Achieve Your Goals

Highest recovery, highest selectivity, and highest reproducibility equal increased certainty. See the difference an mRP-C18 column can make.

To Learn More

- Visit www.agilent.com/chem/bioreagents
- Or go to Agilent's Online Store at www.agilent.com/chem/store
- Or call 1-800-227-9770 (in the U.S. and Canada).
- Or contact your local Agilent office or authorized Agilent distributor.

Information, descriptions and specifications in this publication are subject to change without notice.

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Inquire about custom mRP-C18 columns at affinity_removal@agilent.com



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